Research Article

Molecular Bases of â–Thalassaemia in the Thalassaemic Population of Bhopal.

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Abstract:

Beta- Thalassaemia is a group of heterogeneous recessive disorders common in many parts of the world and one of a major haemoglobinopathy of wide occurrence in the Indian sub-continent. It is distributed to different degrees in different sub-populations. The treatment of this disorder is quite expensive and counseling seems to be the only way for controlling it. Genetic analysis for Beta - Thalassaemia disorder is carried out by Amplification Refractory Mutation System (ARMS) technique. Blood samples of 50 cases of thalassaimia were obtained from patient attending Pediatrics OPD of Gandhi Medical College & Delta Pathology laboratory, Bhopal and were tested. Out of seven common â - thalassaemia mutation, IVS1 [Intra Venous Sequences] nt 5 [nucleotides] (G→C)], IVS1 nt 1 (G→T), Deletion 619 bp (basic pair) and Cap+1(A→C) were found in population of Bhopal in 39.52%, 16.27%, 18.59%, 6.97% respectively. Early detection of thalassaemia is, therefore, important not only from treatment point of view, but also for the prevention by genetic counseling.

Key Words : â - thalassaemia, Central India, Mutation, Genetic counseling, Prenatal diagnosis.

Introduction:

Thalassemias are the most common monogenic gene disorders in the world. â – Thalassaemia is a group of heterogeneous autosomal recessive disorders, where complete absence or reduced synthesis of â – globin chain occurs in haem protein of haemoglobin. Some times the excessive production of á – globin chains leads to its deposition in RBC resulting in less or ineffective erythropoiesis (Weatherall, 1994; Steinber et al, 2001). Patients of thalassemias present with a wide variability of clinical presentation varying from severe forms (â - thalassaemia major) to a very mild or almost symptom less condition. This variability is owing to the presence of a large number of genetic modifiers affecting the disease. In last two decades over 200 types of different mutations have been studied through out the world (Weatherall, 1994). Patients are generally treated with blood transfusions and iron chelation therapy. Pharmacological therapies have varying degrees of success depending on the genetic modifiers of the disease present in the patients (Borgna-Pignatti et al, 2004; Telfer et al, 2006). Studies undertaken to identify all the modifiers that affect â-thalassaemia will lead to more appropriate genetic counseling during prenatal diagnosis and enable targeted and personalized treatment regimen for patients in the future.

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Material & Methods:

Blood samples of 50 cases from patients attending Paediatrics OPD of Gandhi Medical College and Delta Pathology Laboratory, Bhopal, were tested. The samples were either from confirmed cases of Thalassaemia or carrier patients of such cases seeking prenâtal diagnosis. Out of 50 samples, 43 cases were confirmed, rest of the 7 cases remained unidentified. Genomic DNA was extracted using standard methods from intravenous peripheral blood collected in EDTA-coated tubes. Complete blood count was determined using five part cell counter of Sysmax and HbA2 & HbF was determined by HPLC (VARIANT) manufactured by Bio Rcd, France. Mutation detection was carried out by the method of Fortina et al (1992) which is based on the combination of multiplexing and amplification refractory system. Five common mutations were screened by four separate reactions containing either four normal or four mutant primers. Polymerase chain reaction (PCR) mixtures contained 1 *l* g of genomic DNA, 100mM Tris HCl (pH 8.3), 50mM KCl, 100 iM dNTP mixtures, 1.5mM $MgCl_2$ and 0.01 % (w/v) gelatin in a total volume of 50 µl. The mixtures were heated for 5 minutes at 95°C

followed by the addition of four units of *Taq* DNA polymerase. Twenty five PCR cycles of 95°C for 1 minute and combined annealing and extension at 66°C for 2 minutes with the last cycle of 3 minutes at 66°C were carried out. The *â*- globin strip assay kit was used to confirm the results obtained by the multiplex PCR method as well as detecting those mutations not covered by the above method (Newton et al, 1989; Maggio et al, 1993). In this study, PCR amplification was carried out using biotinylated primers, followed by hybridization of the PCR product to a test strip containing allele-specific oligonucleotide probes immobilized as an array of parallel lines. Bound biotinylated sequences were detected using streptavidin phosphatase and color substrate. The amplified genes when separated on Gel electrophoresis, seven distinct bands were obtained out of which four bands were more prominent.

Result & Discussion:

Out of seven common â - Thalassaemia mutations confiremed by Gel electrophorosis (Shaji et al, 2003), 4 different â - Thalassaemia mutations were identified in the present study of randomly selected Thalassaemia patients in Bhopal. They are IVS1 nt 5 (G→C), IVS1 nt 1 (G→T), Del 619 bp. & Cap+1 (A→C) in 39.5%, 16.27%, 18.59 % & 6.97 % respectively in studied group (Fig.1)

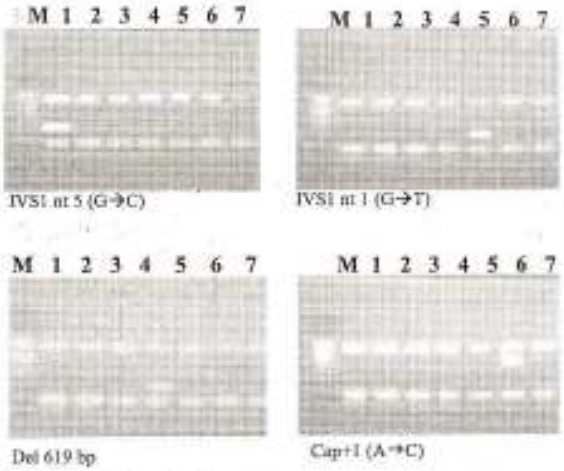

Fig.1: Mutation found in 43 patients.

A similar study was conducted in six different state of India (Varawalla et al, 1991; Table 1). In Punjab the frequency of mutation was 34%, 14.9%, 15.7% and

Table 1: Showing comparative percentage of the mutation found in population of various states of India.(Varawalla et al, 1991) & Present study.

Name of mutation	Punjab	Guj	Maha-rastra	UP	Haryana	Tamil Nadu	Bhopal (Pres. study)
Cap+1 (A→C)	3.7%	-	-	-	-	-	6.97%
IVS1 nt 1 (G→C)	14.9%	16.8%	1.8%	-	-	85.9%	16.27%
IVS1 nt 5 (G→C)	34%	41.1%	59.6%	-	-	-	39.52%
-88 (C→T)	1.6%	-	-	4.9%	1.7%	-	-
619 bp del	15.7%	25.9%	5.3%	3.3%	16.9 %	1.0%	18.57%

3.7%. Apart from these mutation in 1.6% cases an additional mutation -88 (C→T) was also reported. In Gujrat IVS1 nt 1(G→C), IVSI nt 5 (G→C) and 619 bp del was detected in 16.8%, 41.1% & 25.9% cases respectively. In Maharashtra approximately 60% of mutations were of IVS1nt 5(G→C) type. Cap+1 (A→C)mutation was not observed in Maharashtra and Gujrat but is was observed in 6.97% cases in the present study. In contrast to our study in U.P. & Haryana two mutations were observed and they were -88 (C→T) & 619 bp del. IVS1 nt 1 (G→C) mutation was observed in16.27 % cases in the present study while it was 85.9% in Tamil Nadu (Varawalla et al, 1991).

The character wise analysis of the present study revealed that 16.3% (7) cases were compound heterozygous, 51.2% (22) cases were heterozygous and 32.5% (14) were homozygous variety. In another study conducted in India, revealed 60.8% hetrozygous, 32.0% & 34.0 % cases were of different homozygous conditions (Panigrahi et al, 2005, 2006).

Community wise analysis of mutations have shown varying result in the present study. Mutation 619 bp del was found in Sindhi community, IVS1 nt 5 (G→C) in Jain and Cap+1(A→C) in Muslim community. Population wise Punjabis have mix cases of homo and heterozygous IVS1 nt 5 (G→C) mutation in 12%. Hindus too have high ratio of IVS1 nt 5 (G→C) mutation in 51.6%. Sindhi community showed mixed results of two mutations i.e. IVS1 nt 5 (G→C) and Del 619 bp in 12.9%. Rest of the mutation were of mixed frequency.

A study conducted on population of west Bengal reflect that the case of Thalasseamia major is most common among the religious group having inter community marriages as in the case of Muslims (55.26%) and in tribal people it is 29.87% (Sur & Mukhopadhyay 2006).

Population of this region is conscious and willing to accept prenatal diagnosis as a mean of control of thalassaemia.

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